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# Queen control of a key life-history event in a eusocial insect

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**In eusocial insects, inclusive fitness theory predicts potential queen-worker conflict over the timing of events in colony life history. Whether queens or workers control the timing of these events is poorly understood. In the bumblebee *Bombus terrestris*, queens exhibit a 'switch point' in which they switch from laying diploid eggs yielding females (workers and new queens) to laying haploid eggs yielding males. By rearing foundress queens whose worker offspring were removed as pupae and sexing their eggs using microsatellite genotyping, we found that queens kept in the complete absence of adult workers still exhibit a switch point. Moreover, the timing of their switch points relative to the start of egg-laying did not differ significantly from that of queens allowed to produce normal colonies. The finding that bumblebee queens can express the switch point in the absence of workers experimentally demonstrates queen control of a key life-history event in eusocial insects. In addition, we found no evidence that workers affect the timing of the switch point either directly or indirectly via providing cues to queens, suggesting that workers do not fully express their interests in queen-worker conflicts over colony life history.**

**Keywords:** *Bombus*; eusocial insect; life history; queen control; switch point

## 1. INTRODUCTION

The evolution of eusocial societies represents a prime example of a major transition in evolution leading to a new level of individuality [1, 2]. For this reason, colonies of eusocial insects undergo a life history analogous in some respects to that of individual organisms [3]. For example, in annual eusocial Hymenoptera, the change from colony growth (worker production) to reproduction (production of new queens and/or males) is a key life-history event because it represents sexual maturation at the colony level. Inclusive fitness theory predicts potential queen-worker conflict over both sex allocation [4] and the timing of the colony's sexual maturation [5, 6]. The outcomes of such conflicts depend on which party, or parties, within the colony 'control' the relevant trait. Control here refers to any processes, either behavioural or physiological, which allow a given party to affect the trait, including responses to the external environment. By determining the primary sex ratio [7, 8], i.e. the ratio of haploid to diploid eggs laid, queens in the eusocial Hymenoptera potentially exert considerable control in queen-worker conflicts. However, workers may also exert control, through differential rearing of offspring or, as in worker matricide, differential treatment of queens [8, 9]. Additionally, the timing of colony sexual maturation may depend on queens responding to cues provided by workers. Such cues might provide information either on the colony's growth stage or on external environmental conditions such as resource availability [e.g. 10]. They might also provide a means by which workers could indirectly manipulate the timing of colony sexual maturation in their own interests. There has been considerable focus on queen control of the primary sex ratio with respect to sex allocation [8]. But whether such control extends to colony sexual maturation, and whether workers can influence this event, either directly or indirectly, has not been experimentally tested.

The bumblebee *Bombus terrestris* is an annual eusocial insect in which colonies are founded by single queens in spring and produce first workers and then sexuals (new queens and males) before dying out in late summer [11]. Queens exhibit a well-characterized 'switch point' in which, over approximately 8 days, they change from laying diploid eggs yielding females (workers or new queens) to laying haploid eggs yielding males [11, 12]. The switch point typically occurs 2–4 weeks after the eclosion of the first worker (emergence from pupa) [11, 13]. Along with the laying of diploid eggs yielding new queens, which tends to happen shortly beforehand, the switch point marks the colony's sexual maturation [11, 13]. Since potential queen-worker conflict over sex allocation and colony sexual maturation are both present [5, 6], and since queen and workers might benefit from facultatively adjusting the timing of male production to match local conditions [12, 13], it has been hypothesized that the social (colony) environment should affect the switch point. However, previous work has shown little evidence for this, since experimental manipulations of *Bombus* colonies, including doubling worker number in *B. terrestris*, had no significant effect on the timing of male production [14-16]. Queens do not switch to laying haploid eggs through having exhausted their supplies of stored sperm, since post-switch queens retain plentiful, viable sperm [17].

We therefore hypothesized: (1) that *B. terrestris* queens can control the occurrence of the switch point endogenously [cf. ref. 11], i.e. in the complete absence of workers; and (2) that workers do not influence the timing of the switch point directly or indirectly. We tested these hypotheses in a single experiment in which we manipulated the presence of workers within incipient colonies and recorded the occurrence and relative timing of switch points.

## 2. MATERIALS AND METHODS

Post-diapause, mated *Bombus terrestris terrestris* queens ($n$ = 328) were obtained from a commercial supplier in three cohorts (groups received on successive dates) and housed singly in boxes in standard conditions (see electronic supplementary material). The date on which each queen laid her first egg was noted ($n$ = 138 queens). Immediately after each queen had produced her first pupa, i.e. her oldest larva had pupated, she was assigned to a 'social' or 'asocial' treatment ($n$ = 41 queens). The social treatment allowed queens to raise a colony in the normal way. In the asocial treatment, any pupal cocoons were removed before eclosion and discarded. The asocial treatment therefore allowed queens to lay eggs but prevented them from being exposed to any adult offspring. To control for effects of disturbance, equal numbers of cocoons were removed from social queens and then returned.

Every new egg-cell produced by queens in both treatments was removed, censused, sampled for some of its eggs (1–2 eggs removed per egg-cell), resealed and replaced. All removed eggs ($n$ = 1352) were frozen for sexing. Colonies were terminated following either the death of the queen or male eclosion, or, if these events had not occurred, 4–6 months after the beginning of the experiment.

Sampled eggs were sexed using genotyping at five polymorphic microsatellite loci (see electronic supplementary material). Queens were also genotyped. Egg genotypes were scored blindly with respect to sampling date and treatment, and then reconciled with the genotypes of each queen × mate combination, as deduced from the queen and egg genotypes. A queen's switch point was defined as the number of days between her first egg and her first observed haploid egg (as inferred from the egg genotypes). A two-way ANOVA was used to test whether switch point was affected by social versus asocial conditions. All means are expressed ± 1 SD.

## 3. RESULTS

Of 41 queens producing pupae, 17 were assigned to the social treatment and 24 to the asocial treatment. Of these, 10 and 7 queens, respectively, produced sufficient eggs (>5) that could be sexed (see electronic supplementary material) and exhibited a switch point. Among these 'switching queens', the mean switch points of social and asocial queens were 53.0 ± 8.4 and 56.0 ± 14.3 days, respectively (figures 1a, 2). These switch points did not differ significantly (two-way ANOVA, $F_{1, 15} = 0.16$, $p = 0.697$). Furthermore, there was no significant effect of cohort ($F_{2, 14} = 1.71$, $p = 0.216$) on switch point, or significant treatment-cohort interaction ($F_{2, 11} = 3.17$, $p = 0.082$). Switch points of the asocial queens did not differ significantly (Wilcoxon rank sum continuity test, $W = 775$, $n = 177$, $p = 0.239$) from simulated switch points calculated by re-sampling eggs from social queens at sample sizes equivalent to those obtained in asocial queens (see electronic supplementary material). This showed that lower sample sizes of eggs for asocial queens (figure 2) did not bias the switch point estimates. However, a power analysis showed that the minimum detectable difference in the switch points of our samples was 11–12 days (see electronic supplementary material).

Queens that failed to exhibit a switch point laid their last diploid egg significantly earlier than the switch point of switching queens (43.0 ± 13.5 v. 54.2 ± 10.9 days after first egg, respectively; Welch's *t*-test, $t_{15} = 2.36$, $p = 0.032$), suggesting that non-switching queens failed to switch because they had stopped laying eggs before the switch point was reached. Among switching queens, social queens laid significantly more eggs (over 3 times more) before the switch point than asocial queens (Welch's *t*-test, $t_{11} = 4.71$, $p < 0.001$; figure 1b). Asocial queens almost certainly laid fewer eggs through lacking resources and aid supplied by workers, and this, combined with earlier cessation of egg-laying leading to a lower

probability of switching, would account for the smaller proportion of asocial queens that exhibited a switch point relative to social queens.

## 4. DISCUSSION

We found that queens of the bumblebee *Bombus terrestris* reared in asocial conditions, in which they were never exposed to their adult worker offspring, switched to laying haploid, male eggs as did social, control queens allowed to produce adult worker offspring in the normal way. This result experimentally demonstrates that the switch point, which represents a key life-history event in colony development, can be controlled by queens endogenously. Moreover, we found no significant difference in the timing of the switch point between social queens and asocial queens. However, our data do not preclude an influence of workers on the timing of the switch point within the limits specified by the power analysis. Nonetheless, we found no evidence that workers exert a large influence over colony sexual maturation either directly or indirectly, suggesting that workers do not express their interests fully in queen-worker conflicts over colony life history.

An endogenous mechanism of determining the switch point permits *B. terrestris* queens to exercise control in kin-selected conflicts with workers over sex allocation [18] and colony sexual maturation [5, 6]. However, the switch point may still be responsive to external cues. For example, Duchateau *et al*. [12] found that *B. terrestris* queens undergoing longer periods of diapause exhibited earlier switch points, suggesting that queens use 'personal' cues stemming from their pre-founding or founding experience and/or their own quality to modulate the timing of the switch point.

We hypothesize that queens use such personal cues to initiate an internal interval timer [19] that 'counts down' to the switch point. This hypothetical timer appears to be independent of periodism in light levels and temperature, since queens were kept in darkness at constant temperature. Queens do not lay a standard number of diploid eggs before switching to laying haploid eggs, since we found that social queens laid significantly more eggs than asocial queens before switching even though they switched at the same relative date. Overall, the proximate mechanism underlying endogenous queen control of the switch point in *B. terrestris* is unknown and deserves future investigation.

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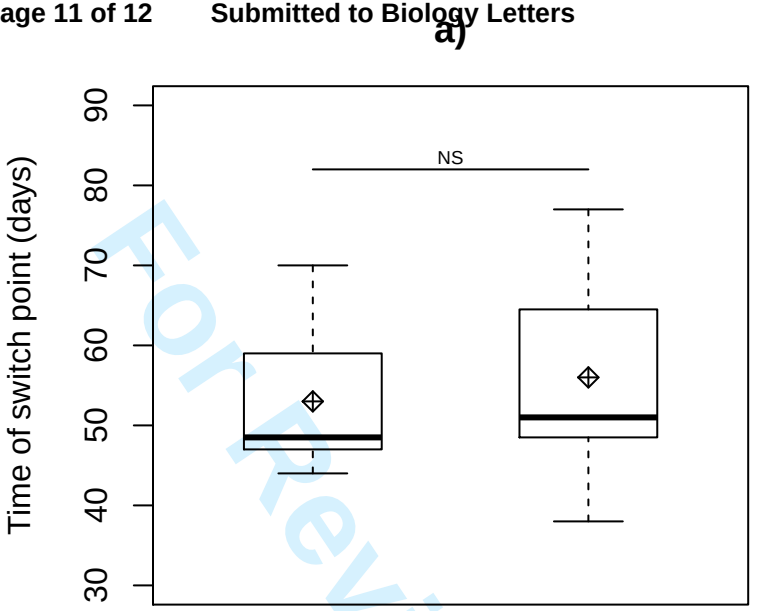
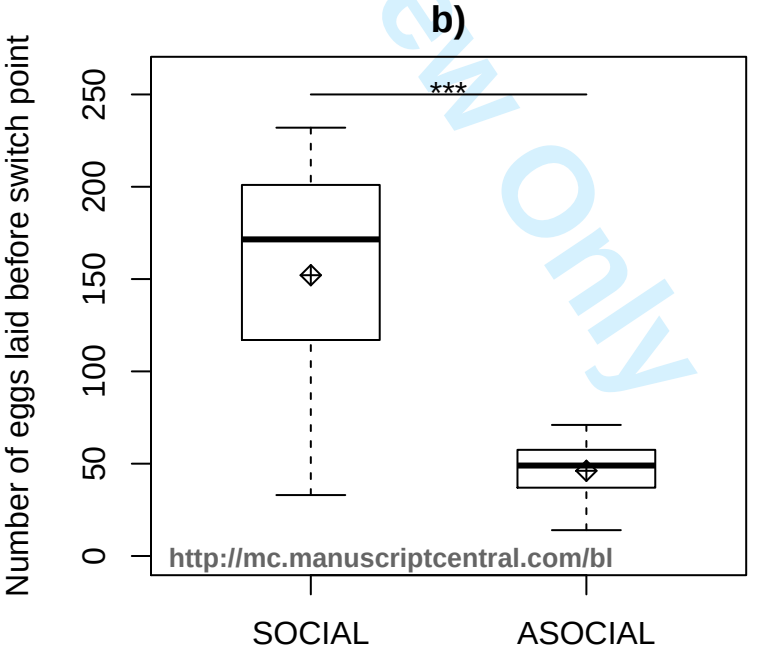
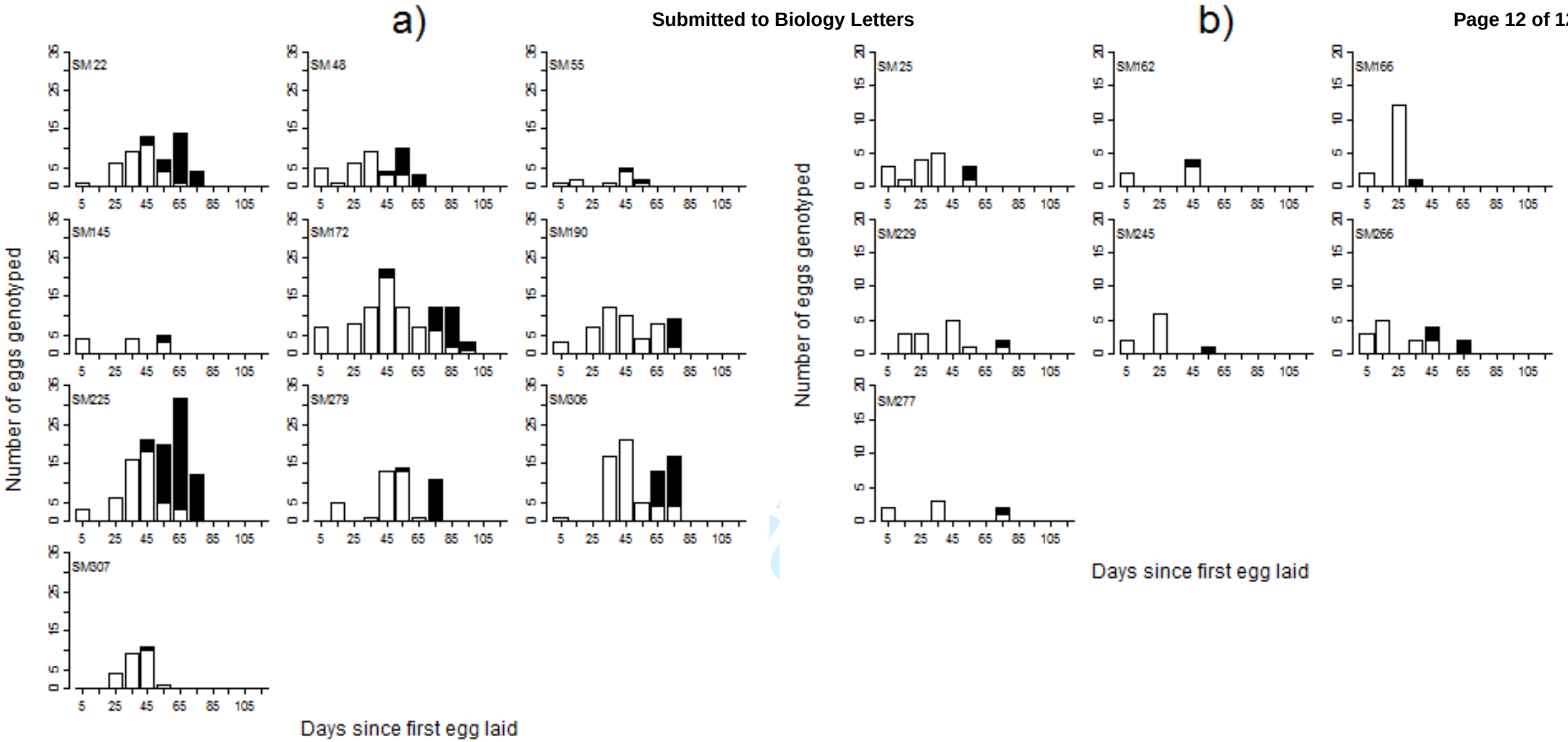
Figure legends

Figure 1. (a) Time of the switch point (days from queen's first egg) and (b) number of eggs laid before the switch point in *Bombus terrestris* queens in social ($n$ = 10) and asocial ($n$ = 7) treatments. Diamonds, means; thick horizontal bars, medians; boxes, interquartile range; whiskers, range. (a) NS, not significant (ANOVA); (b), ***, $p < 0.001$ (Welch's $t$-test).

Figure 2. The numbers of sampled diploid and haploid eggs laid by *Bombus terrestris* queens over time in (a) social and (b) asocial treatments ($n$ = 10 and 7 queens, respectively). Each plot represents a separate queen (id code in upper left corner). White shading, diploid eggs; black shading, haploid eggs; total heights of bars, number of eggs genotyped in each time bloc.

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Days since first egg laid